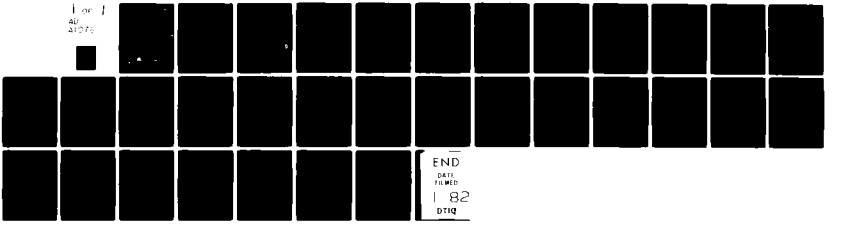
AD-A107 628 LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANC--ETC F/G 6/20
THE MUTAGENIC POTENTIAL OF: (E)-1,2,3,4-TETRAHYDRO-6-METHYL-1(2--ETC(U)
SEP 81 L J SAUERS, F R PULLIAM, J T FRUIN
UNCLASSIFIED LAIR-109 NL



LEVEL II

2

INSTITUTE REPORT NO. 109

THE MUTAGENIC POTENTIAL OF:

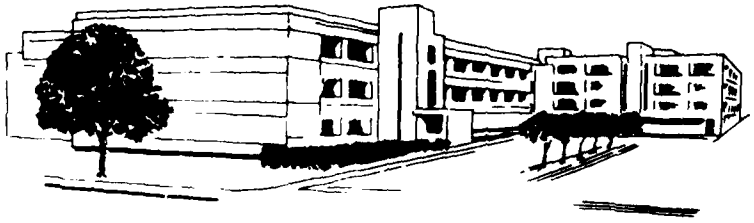
(É)-1,2,3,4-tetrahydro-6-methyl-1(2-methyl-1-oxo-2-butenyl)quinoline
1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline
50% DEET, 25% Dow Corning 200 fluid, in isopropanol .

LEONARD J. SAUERS, BA, SP5
FREDDICA R. PULLIAM, BS, SSG
and
JOHN T. FRUIN, DVM, PhD, LTC VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

SEPTEMBER 1981

Toxicology Series 20



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Toxicology Series 20

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James A. Marshall 23 Sept 87
(Signature and date)

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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Mutagenicity, Toxicology, Ames Assay, (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl)quinoline; 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline; 50% DEET, 25% Dow Corning 200 Fluid, in isopropanol;

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl)quinoline (CHR5); 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline (CHR6); 50% DEET, 25% Dow Corning 200 Fluid, in isopropanol (CHF1); was assessed using the Ames/Salmonella Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538, were exposed to 1 ul/plate through 3.2×10^{-4} ul/plate doses of CHR5 and CHR6 and 0.1 ul/plate through 3.2×10^{-5} ul/plate doses of CHF1. No evidence of mutagenic activity was observed.

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ABSTRACT

The mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl)quinoline(CHR5*); 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline (CHR6*); 50% DEET, 25% Dow Corning 200 Fluid, in isopropanol (CHF1*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to 1 ul/plate through 3.2×10^{-4} ul/plate doses of CHR5 and CHR6 and 0.1 ul/plate through 3.2×10^{-5} ul/plate doses of CHF1. No evidence of mutagenic activity was observed.

* Code number for compound.

PREFACE

AMES ASSAY REPORT:

SUBSTANCE	CODE NO.
(E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-1-butenyl)quinoline	CHR5
1,2,3,4-tetrahydro-6-mthyl-1-(3-methyl-1-oxo-2-butenyl)quinoline	CHR6
50% DEET, 25% Dow Corning 200 Fluid in isopropanol	CHF1

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Division of Cutaneous Hazards
Letterman Army Institute of Research

PROJECT: More Effective Topical Repellents Against Disease Bearing Mosquitoes 3M62272A810

GLP STUDY NUMBER: 81017

STUDY DIRECTOR: LTC John T. Fruin D.V.M.,PhD.
CO-PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, B.S.
SP5 Leonard J. Sauers, B.A.

RAW DATA: A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test substances were provided by sponsor. Chemical, analytical, stability, purity, etc. data are available from the sponsor.

PURPOSE: To determine the mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl)quinoline; 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline; 50% DEET, 25% Dow Corning 200 Fluid, in isopropanol, by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

ACKNOWLEDGMENTS

The authors wish to thank SP4 Thomas Kellner, BA; SP4 Larry Mullen, BS; and John Dacey for assistance in performing the research.

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study, GLP number 81017, described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.

Freddica R. Pulliam 6 Jul 81
FREDDICA R. PULLIAM, BS Date
SSG
Co-Investigator

John T. Fruin July 81
JOHN T. FRUIN, DVM, PhD Date
LTC, VC
Study Director

Leonard J. Sauers 9 July 81
LEONARD J. SAUERS, BA Date
SP5
Co-Investigator

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

22 July 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81017 the following inspections were made:

1000 hr, 5 June 1981
1300 hr, 5 June 1981

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 1981 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to *in vivo* tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of [illegible] strains of Salmonella typhimurium that are unable to [illegible] histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained [illegible] from Dr. Ames, University of California, Berkeley, prepared [illegible] maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains were used 16 *hours (maximum)* after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000- fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous

revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, all the test substances will be referred to by their respective code numbers:

Substance	Code No
(E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl)quinoline	CHR5
1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline	CHR6
50% DEET, 25% Dow Corning 200 Fluid, in Isopropanol	CHF1

On 3 June 1981, the Toxicity Level Determination was performed on the 3 test chemicals. For this experiment, all sterility, strain

verification, positive and negative controls were normal (Table 1). The plates containing the initial dilution showed slight growth for CHR5, normal growth for CHR6, and no growth for CHF1 (Tables 2A-2C). It was decided to use 0.1 ul/plate as the starting point for CHF1 and 1 ul/plate for the other test substances.

On 2 July 1981, The Ames Assay was run on the 3 test compounds. All sterility and strain verification controls were normal (Table 3). Unexpected results were observed in response to positive control chemical DMBA for all the strains. Expected results were seen in response to MNNG, AF, and BP, which validates our data since these other controls function through similar mechanisms. The negative controls were normal (Table 4).

No mutagenic activity was observed in response to test chemical CHR5 (Table 5A). One isolated incidence of mutagenic activity was seen for CHR6. This occurred at the 0.008 ul/plate dose for nonactivated TA 1537. No dose response was observed (Table 5B). A doubling of the spontaneous revertant rate was noticed in response to CHF1 at the 1.6 x 10-4 ul/plate level for nonactivated TA 1537 and nonactivated TA 1538, at the 0.02 ul/plate dose. No dose response was seen in either case (Table 5C).

The MUTAR values are listed in Tables 6A-6C. All calculations resulted in expected responses except for nonactivated TA 1538 at the 0.02 ul/plate dose level of CHF1 (Table 6C).

CONCLUSION

The results showed several isolated incidences of a doubling of the spontaneous reversion rate. It is in the opinion of the Ames Assay Laboratory at the University of California, Berkeley, that even though a doubling had occured, one cannot declare mutagenicity unless an obvious dose response is seen (Maron D., Ames Assay Laboratory, University of California, Berkeley, 30 March 1981). Therefore on the basis of the Ames Test, compounds CHR5, CHR6, and CHF1 are not mutagenic at the levels tested.

RECOMMENDATION

We recommend that candidate insect repellents CHR5, CHR6, and CHF1 be tested further with other toxicological assays if efficacy tests show these compounds to be promising repellents.

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4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties. J Biol Chem 218: 97-106, 1956

5. COMMONER, B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976

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TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	15.46 mm	NG	+
1537	NG	NG	NG	14.71 mm	NG	+
WT	G	NA	G	NA	NG	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MCA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) CHR5-NG (b)CHR6-NG (c)CHF1-NG (d) NA (e) NA

G = Growth NC = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 - Average - 140 Positive Control - MNNG - 1612

(1) + = expected response - = unexpected response

Study Number: 81017 Date: 3 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 2A

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR5 Substance dissolved in: ETOH

Study Number: 81017 Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 ul/plate	122	168	150	147	ST
0.1 ul/plate	145	167	188	167	NL
10^{-2} ul/plate	132	121	167	140	NL
10^{-3} ul/plate	205	175	145	175	NL
10^{-4} ul/plate	137	147	129	138	NL
10^{-5} ul/plate	160	125	120	135	NL
10^{-6} ul/plate	150	180	168	166	NL
10^{-7} ul/plate	152	179	199	177	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 2B

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR6 Substance dissolved in: ETOH

Study Number: 81017 Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 ul/plate	107	116	122	115	NL
0.1 ul/plate	88	97	111	99	NL
10^{-2} ul/plate	111	90	104	102	NL
10^{-3} ul/plate	120	121	105	115	NL
10^{-4} ul/plate	131	144	115	130	NL
10^{-5} ul/plate	143	134	137	138	NL
10^{-6} ul/plate	134	162	135	144	NL
10^{-7} ul/plate	165	125	143	144	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 2C

TOXICITY LEVEL DETERMINATION

Substance assayed: Code CHF1 Substance dissolved in: ETOH

Study Number: 81017 Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 ul/plate	Toxic	Toxic	Toxic	Toxic	NG
0.1 ul/plate	85	86	110	94	NL
10^{-2} ul/plate	136	119	102	119	NL
10^{-3} ul/plate	117	134	150	134	NL
10^{-4} ul/plate	123	119	158	133	NL
10^{-5} ul/plate	156	128	155	146	NL
10^{-6} ul/plate	144	158	115	139	NL
10^{-7} ul/plate	141	177	133	150	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	14.55 mm	NG	+
100	NG	G	NG	16.0 mm	NG	+
1535	NG	NT	NG	15.80 mm	NG	+
1537	NG	25.25 mm	NG	16.47 mm	NG	+
1538	NG	NT	NG	15.42 mm	NG	+
WT	G	NT	G	NT	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NT

S-9 Mix Initial: NG End: NG Nutrient Broth: NT

Test Compound (a) CHF1=NG (b) CHR5=NG (c) CHR6=NG (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 81017 By: Dacey, Pulliam, Kellner

Date: 2 July 1981

(1) + = expected response
- = unexpected response

TABLE 4

POSITIVE CONTROL REVERTANT RATE AND SPONTANEOUS REVERTANT RATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
AF	2 ug/plate	yes	(360,413,270) (348)	(243,426,215) (295)			(55,Tox,Tox) (55)
BP	2 ug/plate	yes	(100,68,52) (73)	(446,267,271) (328)		(53,26,22) (34)	(Tox,Tox,Tox) Tox)
DMBA	20 ug/plate	yes	(19,38,20) (26)	(170,131,166) (156)		(10,18,15) (14)	(15,9,13) (12)
MNNG	2 ug/plate	no		(985,1041,1050) (1025)			
	20 ug/plate	no			(580,Tox,Tox) (580)		
Spontaneous Revertant Test							
	before	yes	(30,14,24)	(122,101,122)	(13,16,15)	(12,11,7)	(10,14,27)
	after	yes	(12,20,8) (18)	(77,69,87) (96)	(14,8,5) (12)	(3,11,4) (8)	(8,NG*,NG*) (15)
	before	no	(12,23,9)	(89,70,85)	(15,7,13)	(9,2,5)	(7,9,16)
	after	no	(9,18,7) (11)	(117,71,75) (85)	(11,17,15) (13)	(3,3,0) (4)	(11,1,NG*) (9)

Study Number: 81017

Date: 2 July 1981 By: Dacey, Kellner, Pulliam

* No background lawn

TABLE 5A
NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR5	1 ul/plate	no	(10,16,7) (11)	(54,60,58) (57)	(12,9,12) (11)	(7,4,6) (6)	(6,5,8) (6)
		yes	(17,28,16) (20)	(109,119,92) (107)	(19,17,9) (15)	(3,8,0) (4)	(24,19,18) (24)
CHR5	0.2 ul/plate	no	(2,8,4) (5)	(63,83,82) (76)	(7,13,7) (9)	(2,5,3) (3)	(6,8,5) (6)
		yes	(20,10,16) (15)	(59,68,79) (69)	(12,13,20) (15)	(7,6,3) (5)	(23,22,9) (18)
CHR5	0.04 ul/plate	no	(2,14,7) (8)	(87,64,68) (73)	(22,8,9) (13)	(3,4,9) (5)	(15,11,11) (12)
		yes	(27,24,24) (25)	(79,72,71) (74)	(16,13,21) (17)	(9,3,3) (5)	(22,20,21) (21)

-continued

Study Number: 81017 Date: 2 July 1981 By: Dacey, Kellner, Pulliam

TABLE 5A, concluded

NUMBER OF REVERTANTS/I LATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR5	0.008 ul/plate	no	(20,8,12) (13)	(98,60,71) (76)	(15,8,9) (11)	(6,3,3) (4)	(9,6,11) (9)
		yes	(22,12,3) (12)	(84,106,95) (95)	(13,23,12) (16)	(8,8,6) (7)	(15,15,12) (14)
CHR5	0.0016 ul/plate	no	(11,15,11) (12)	(62,87,78) (76)	(19,12,13) (15)	(9,3,6) (6)	(2,8,7) (6)
		yes	(24,14,17) (18)	(66,57,55) (59)	(12,13,4) (10)	(5,8,4) (6)	(5,5,2) (4)
CHR5	0.00032 ul/plate	no	(9,12,16) (12)	(81,79,61) (74)	(10,12,14) (12)	(3,6,2) (4)	(8,7,8) (8)
		yes	(36,27,20) (28)	(110,80,94) (95)	(11,13,11) (12)	(7,3,6) (5)	(19,9,18) (15)

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TABLE 5B

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR6	1 ul/plate	no	(Tox,Tox,Tox) (Tox)	(Tox,Tox,Tox) (Tox)	(Tox,Tox,Tox) (Tox)	(Tox,Tox,Tox) (Tox)	(Tox,Tox,Tox) (Tox)
		yes	(Tox,Tox,23) (Tox)	(55,Tox,66) (61)	(Tox,Tox,Tox) (Tox)	(Tox,Tox,Tox) (Tox)	(Tox,Tox,Tox) (Tox)
CHR6	0.2 ul/plate	no	(15,17,11) (14)	(88,26,72) (62)	(6,12,6) (8)	(1,8,3) (4)	(9,8,9) (9)
		yes	(23,10,21) (18)	(105,126,114) (115)	(26,19,18) (21)	(4,4,2) (3)	(3,8,1) (4)
CHR6	0.04 ul/plate	no	(14,10,10) (15)	(78,66,88) (77)	(6,15,6 (9)	(3,5,2) (3)	(5,4,2) (3)
		yes	(22,30,20) (24)	(107,106,85) (99)	(14,15,13) (14)	(8,5,3) (5)	(17,19,25) (20)
CHR6	0.008 ul/plate	no	(21,13,12) (15)	(76,87,92) (85)	(6,22,5) (11)	(17,6,5) (9)	(10,3,11) (10)
		yes	(20,18,12) (17)	(67,72,82) (74)	(12,8,9) (10)	(4,2,8) (5)	(19,16,7) (14)

-continued

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TABLE 5B, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHR6	0.0016 ul/plate	no	(5,14,9) (9)	(57,73,70) (67)	(6,15,12) (11)	(1,17,6) (8)	(6,4,2) (4)
		yes	(19,13,13) (15)	(78,78,61) (72)	(13,9,8) (10)	(5,5,6) (5)	(20,12,28) (20)
CHR6	0.00032 ul/pl.	no	(5,19,10) (11)	(81,56,68) (68)	(4,12,10) (9)	(2,6,3) (4)	(10,12,10) (11)
		yes	(11,10,20) (14)	(52,81,65) (66)	(8,6,6) (7)	(4,8,2) (5)	(10,19,14) (14)

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TABLE 5C

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHF1	0.1 ul/plate	no	(21,16,12) (16)	(141,106,74) (107)	(15,10,17) (14)	(8,5,7) (7)	(38,13,4) (18)
		yes	(32,27,25) (28)	(150,123,110) (128)	(22,22,18) (21)	(8,3,16) (9)	(11,13,33) (19)
CHF1	0.02 ul/plate	no	(21,23,20) (21)	(110,111,113) (111)	(12,30,18) (20)	(3,4,5) (4)	(28,32,31) (30)
		yes	(10,20,8) (13)	(70,70,88) (76)	(16,13,12) (14)	(10,6,13) (10)	(5,11,6) (7)
CHF1	0.004 ul/plate	no	(3,4,5) (4)	(73,64,66) (68)	(9,9,7) (8)	(3,4,4) (3)	(16,11,2) (10)
		yes	(17,21,17) (18)	(95,81,64) (80)	(16,12,11) (13)	(6,2,5) (4)	(20,23,16) (20)
CHF1	0.0008 ul/pl.	no	(5,12,4) (7)	(64,56,65) (62)	(9,8,11) (9)	(9,10,6) (8)	(3,5,8) (5)
		yes	(27,36,24) (29)	(99,93,86) (93)	(16,18,21) (18)	(9,4,4) (6)	(14,14,17) (15)

-continued

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TABLE 5C, concluded
NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CHF1	0.00016 ul/pl.	no	(6,15,19) (13)	(98,93,93) (95)	(14,22,11) (16)	(11,12,9) (11)	(8,15,17) (13)
		yes	(16,18,25) (20)	(103,101,126) (110)	(22,12,18) (17)	(9,8,2) (6)	(19,20,18) (19)
CHF1	0.000032 ul/pl.	no	(24,22,14) (20)	(123,111,102) (112)	(18,15,8) (14)	(9,3,6) (6)	(13,7,11) (10)
		yes	(23,26,20) (23)	(81,66,120) (89)	(12,21,19) (17)	(6,3,6) (5)	(12,4,0) (5)

Study Number: 81017 Date: 2 July 1981 By: Dacey, Kellner, Pulliam

TABLE 6A

MUTAGENIC ACTIVITY RATIO

Substance Assayed: CHR5 Dissolved in: ETOH

Study Number: 81017 Date: 6 Aug 81 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1 ul/plate	TA 98	0.09	*	0.008 ul/pl.	TA 1535	0.36	*
0.2 ul/plate	TA 98	*	*	0.0016 ul/pl.	TA 1535	*	0.13
0.04 ul/plate	TA 98	0.30	*	0.00032 ul/pl.	TA 1535	*	*
0.008 ul/plate	TA 98	*	0.11				
0.0016 ul/pl.	TA 98	*	0.05	1 ul/plate	TA 1537	*	0.33
0.00032 ul/pl.	TA 98	0.43	0.05	0.2 ul/plate	TA 1537	*	*
				0.04 ul/plate	TA 1537	*	0.17
1 ul/plate	TA 100	0.1	*	0.008 ul/plate	TA 1537	*	*
0.2 ul/plate	TA 100	*	*	0.0016 ul/pl.	TA 1537	*	0.33
0.04 ul/plate	TA 100	*	*	0.00032 ul/pl.	TA 1537	*	*
0.008 ul/plate	TA 100	*	*				
0.0016 ul/pl.	TA 100	*	*	1 ul/plate	TA 1538	0.5	*
0.00032 ul/pl.	TA 100	*	*	0.2 ul/plate	TA 1538	0.17	*
				0.04 ul/plate	TA 1538	0.33	0.23
1 ul/plate	TA 1535	0.27	*	0.008 ul/pl.	TA 1538	*	*
0.2 ul/plate	TA 1535	0.27	*	0.0016 ul/pl.	TA 1538	*	*
0.04 ul/plate	TA 1535	0.45	*	0.00032 ul/pl.	TA 1538	*	*

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR, or a zero MUTAR

TABLE 6B

MUTAGENIC ACTIVITY RATIO

Substance Assayed: CHR6 Dissolved in: ETOH

Study Number: 81017 Date: 3 August 81 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1 ul/plate	TA 98	*	*	0.008 ul/plate	TA 1535	*	*
0.2 ul/plate	TA 98	*	0.16	0.0016 ul/pl.	TA 1535	*	*
0.04 ul/plate	TA 98	0.26	0.21	0.00032 ul/pl.	TA 1535	*	*
0.008 ul/plate	TA 98	*	0.21				
0.0016 ul/pl.	TA 98	*	*	1 ul/plate	TA 1537	*	*
0.00032 ul/pl.	TA 98	*	*	0.2 ul/plate	TA 1537	*	*
				0.04 ul/plate	TA 1537	*	*
1 ul/plate	TA 100	*	*	0.008 ul/plate	TA 1537	*	0.83
0.2 ul/plate	TA 100	0.18	*	0.0016 ul/pl.	TA 1537	*	0.67
0.04 ul/plate	TA 100	0.03	*	0.00032 ul/pl.	TA 1537	*	*
0.008 ul/plate	TA 100	*	*				
0.0016 ul/pl.	TA 100	*	*	1 ul/plate	TA 1538	*	*
0.00032 ul/pl.	TA 100	*	*	0.2 ul/plate	TA 1538	*	*
				0.04 ul/plate	TA 1538	0.17	*
1 ul/plate	TA 1535	*	*	0.08 ul/plate	TA 1538	*	0.08
0.2 ul/plate	TA 1535	0.8	*	0.0016 ul/pl.	TA 1538	0.28	*
0.04 ul/plate	TA 1535	0.18	*	0.00032 ul/pl.	TA 1538	*	0.15

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR, or a zero MUTAR

TABLE 6C

MUTAGENIC ACTIVITY RATIO

Substance Assayed: CHF1 Dissolved in: ETOH

Study Number: 81017 Date: 3 August 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
0.1 ul/plate	TA 98	0.43	0.26	0.0008 ul/pl.	TA 1535	0.54	*
0.02 ul/plate	TA 98	*	0.53	0.00016 ul/pl.	TA 1535	0.45	0.2
0.004 ul/plate	TA 98	*	*	0.000032 ul/pl	TA 1535	0.45	0.07
0.0008 ul/pl.	TA 98	0.47	*				
0.00016 ul/pl.	TA 98	0.09	0.11	0.1 ul/plate	TA 1537	0.15	0.5
0.000032 ul/pl.	TA 98	0.21	0.47	0.02 ul/plate	TA 1537	0.29	*
				0.004 ul/plate	TA 1537	*	*
0.1 ul/plate	TA 100	0.3	0.24	0.0008 ul/pl.	TA 1537	*	1.17
0.02 ul/plate	TA 100	*	0.28	0.00016 ul/pl.	TA 1537	*	0.33
0.004 ul/plate	TA 100	*	*	0.000032 ul/pl	TA 1537	*	0.33
0.0008 ul/pl.	TA 100	*	*				
0.00016 ul/pl.	TA 100	0.13	0.11	0.1 ul/plate	TA 1538	0.22	0.68
0.000032 ul/pl.	TA 100	*	0.29	0.02 ul/plate	TA 1538	*	1.59
				0.004 ul/plate	TA 1538	0.28	0.08
0.1 ul/plate	TA 1535	0.8	0.07	0.0008 ul/pl.	TA 1538	*	*
0.02 ul/plate	TA 1535	0.18	0.47	0.00016 ul/pl.	TA 1538	0.22	0.3
0.004 ul/plate	TA 1535	0.09	*	0.000032 ul/pl.	TA 1538	*	0.08

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR, or a zero MUTAR

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